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Production of high-molecular-weight ribonuclease Bsn from the recombinant strain of Bacillus subtilis

Kharitonova M., Znamenskaya L., Leshchinskaya I.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

Background: Ribonucleases (RNases) can be used in both basic and clinical sciences, e.g. in research on developmental processes or on antiviral and antitumor therapy. RNases have great potential as therapeutic entities. On the basis of new ribonucleases new medications can be created. Bacilli synthesize two types of secretory ribonucleases, the well-studied low-molecula--weight ribonucleases and high-molecular-weight ribonucleases. Only two RNases of the second type have so far been described: RNase Bsn from B. subtilis and binase II from B. intermedius. Materials/Methods: The activity of ribonucleases was determined from the amount of the acid-soluble products of RNA hydrolysis. The cultivation media were optimized for maximum RNase production in terms of the experimental factorial design B2 using BIOPT software. Results: Our investigation of a novel secretory ribonuclease, the Bacillus subtilis RNase Bsn expressed in the recombinant B. subtilis strain 168, showed that it is synthesized in the growth retardation phase, when inorganic phosphate is exhausted in the medium. The biosynthesis of Bsn was found to be suppressed by inorganic phosphate in the medium and activated by small amounts of the transcriptional inhibitor actinomycin D. Conclusion: Our results show that the biosynthesis of the novel secretory ribonuclease Bsn in recombinant strain Bacillus subtilis 168 is subject to negative regulation by inorganic phosphate, and is activated by small doses of actinomycin D. The stimulating effect of this antibiotic is well pronounced during the active synthesis of ribonucleases, but insignificant when ribonuclease synthesis is inhibited by Pi.

Keywords

Actinomycin D, Bacillus subtilis, Biosynthesis, Phosphate starvation, RNase Bsn